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Bovine genetic resistance effects on biological traits of *Rhipicephalus* (*Boophilus*) *microplus*

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ABSTRACT

This study aimed to verify the influence of bovine genetic resistance on biological traits of the *Rhipicephalus* (*Boophilus*) *microplus* tick. Genetic resistance or susceptibility was determined according to breeding values for tick counts, predicted using a dataset of 9007 Hereford and Braford (Hereford × Zebu) bovines naturally infested and raised under extensive production systems in southern Brazil. From a total of 974 Braford heifers born in 2008, 20 were classified as genetically tick-resistant and 20 classified as genetically tick-susceptible, and used to obtain the ticks samples used in this study. The 40 heifers were exposed to four subsequent artificial infestations with approximately 20,000 larvae at 14-day intervals. From the 19th to 23rd day of each infestation tick counts were performed on the left body side of the heifers. Engorged ticks were manually collected on the day of highest observed burden after each infestation. Tick counts on susceptible heifers were 5.5, 10.5, 11.1 and 6.9 times larger than on resistant heifers, respectively, after the first, second, third and fourth artificial infestations. In the third infestation, ticks from resistant heifers showed lower egg production index ($P < 0.0001$) than ticks from susceptible heifers. In the fourth infestation, ticks from susceptible group showed higher egg mass weight ($P < 0.05$) and nutrient index ($P < 0.0001$) than ticks from resistant heifers. Tick initial weights showed a positive association with egg production index in susceptible heifers ($P < 0.05$) and a negative association in the resistant group ($P < 0.05$), suggesting a host defense mechanism that reduces the conversion efficiency of ingested blood to eggs in engorged ticks from resistant cattle. This shows that bovine genetic tick resistance, in addition to affecting the number of ticks carried by the animals, also affected the egg mass weight, egg production and nutrient indexes of ticks. The results of the present study imply that the selection of resistant animals could be used as a strategic tool for tick control in production systems, reducing infestation levels on cattle and environment.

1. Introduction

The *Rhipicephalus* (*Boophilus*) *microplus* tick infestation is one of the main environmental challenges and has major impact on cattle production systems in tropical and subtropical countries (Mendes et al., 2011). Anemia,

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bodyweight loss, decreased milk production, transmission of diseases and high costs of chemical control are some of the direct and indirect causes of economic losses caused by this parasite (Jongejan and Uilenberg, 2004; Jonsson, 2006). Furthermore, the increasing development of acaricide resistance by tick populations and the possibility of environmental contamination by the chemical treatments have stimulated several researchers to investigate alternative control methods (George et al., 2004; de la Fuente et al., 2007). Selection of cattle to increase herd frequency of favorable alleles for genes related to tick resistance has been indicated as an alternative control strategy with reduced use of chemicals (Frisch et al., 2000).

Genetic variability for tick counts in cattle has been reported between and within breeds, and it is well documented that *Bos indicus* cattle are more resistant to tick infestation than *Bos taurus* (Fraga et al., 2003; Henshall, 2004; Prayaga and Henshall, 2005; Regitano et al., 2008). Differences in immune response profiles and polymorphisms in genes responsible for encoding cellular or humoral immune factors have been associated with resistance or susceptibility phenotypes (Acosta-Rodríguez et al., 2005; Martinez et al., 2006; Piper et al., 2010; Porto Neto et al., 2013). Such studies are essential to support the possibility of genetic progress through selection of animals classified as genetically resistant and for the development of anti-tick vaccines.

Besides influencing tick burden, Wagland (1975) reported that cattle genetic resistance also affected the length of feeding and detachment weight of female engorged ticks from Brahman and Shorthorn cattle. Studying repeatedly infested Hereford calves, Barriga et al. (1993) suggested that genetic differences among animals affect biological traits of *R.* (*B.*) *microplus* engorged on seemingly homogeneous individuals (same breed, gender, age and management).

Although the counting of engorged female ticks after natural or artificial infestations has been effectively used to measure genetic tick resistance in cattle, studies that also investigate possible effects of host resistance on tick functions may contribute to the understanding of mechanisms involved in host × parasite interaction. Therefore, the objective of this study was to evaluate the influence of cattle genetic resistance on biological traits of engorged *R.* (*B.*) *microplus* ticks from artificially infested Braford heifers classified as tick-resistant or tick-susceptible.

2. Materials and methods

2.1. Animals and determination of genetic resistance to ticks

Classification of animals as tick-resistant or tick-susceptible was based on their predicted breeding values for tick counts. The (co)variance components and genetic parameters necessary to calculate breeding values were obtained from 9007 records of Hereford and Braford (Hereford × Zebu) cattle raised in extensive production systems in southern Brazil and belonging to the Delta G Connection Genetic Improvement Consortium (Delta G Connection, 2007). At the beginning of this work, a historical dataset was available including counts of the number of engorged female ticks on the inner hind leg region of 6709 naturally infested bovines born between 2001 and 2007, with one count per animal. A second dataset was collected from the same population during this study and contained information of two or three subsequent tick counts on the whole left body side of 2298 naturally infested bovines born in 2008, totaling 6607 count records. The counts were performed during an extended yearling evaluation period, with an average animal age of 522 ± 66 days. The pedigree information was composed of 17,252 records, including base animals with unknown parents.

The two datasets were jointly analyzed using a bivariate animal model (Henderson, 1984). Counts at the inner hind leg region and on the left body side were considered as different but genetically correlated traits, such that historical records contributed to the estimation of breeding values for current animals through their common ancestry. The statistical model included: the fixed effect of contemporary groups (animals from the same farm, sex, year and season of birth, management group, and date of tick count); the linear covariate effects of breed composition and heterozygosity; the linear and quadratic covariate effects of animal age; and, the random effects of breeding value and residuals. Contemporary groups with less than five individuals and counts that were 3.5 standard deviation above or below the mean tick count of their contemporary group were excluded previously from the analyzes. Tick counts were transformed by applying a base 10 logarithmic function to the observed value + 1. All parameters in the model were jointly estimated using Bayesian inference through the INTERGEN software (Cardoso, 2010).

From a total of 974 Braford heifers (breed composition between ½ Hereford + ½ Zebu and ¾ Hereford + ¼ Zebu) born in the Delta G herds during 2008, 22 classified as extremely resistant and 21 classified as extremely susceptible were selected for this study. The criteria used to define the resistant group included heifers with tick count breeding value within the lowest 10% (<−0.08), average standard deviation of their counts in relation to the contemporary group mean over the subsequent evaluations also within the lowest 10% (<−1.28), and that were always at least 1.0 standard deviation (SD) below the mean of their contemporary group in each count. Heifers with breeding values within the highest 10% (>0.10), average standard deviation also within the highest 10% (>1.28) and always 1.0 SD above the mean for their contemporary group in each count were considered the susceptible group.

2.2. Artificial infestations

Selected heifers were transferred from their original farms to Embrapa Southern Region Animal Husbandry Center, in the city of Bagé, Rio Grande do Sul state, Brazil (latitude 31°19′S, longitude 54°06′W and altitude 212 m), where artificial infestations were performed. Before the experiment, all heifers were treated with a commercial amitraz acaricide and maintained in a tick-free pasture during three months. Artificial infestations used *R.* (*B.*) *microplus* larvae between 10 and 15 days old from a strain susceptible to common acaricides and free of *Babesia* sp.

and *Anaplasma* sp., maintained by Embrapa Ectoparasitology Laboratory. Aliquots of 1 g of eggs (about 20,000 larvae) were placed in syringes and incubated at 27 ± 1 °C and relative humidity at 80 ± 10% for larvae hatching. Four successive artificial infestations were performed, at 14-day intervals, between February 9 and March 23, 2011, through the distribution of larvae along bovine dorsal region. The heifers did not receive any further acaricide treatment until the end of the experimental period.

In the first and third infestations, heifers were treated with 1.2 mg kg^{-1} imidocarb dipropionate (Imizol®) to prevent cases of babesiosis and anaplasmosis that could be transmitted by natural tick infestations from the experimental pasture where the animals were kept after the first infestation. To monitor environmental infestations, only 40 animals were artificially infested (20 from the resistant group and 20 from the susceptible group), and three heifers (two from the resistant group and one from the susceptible group) were maintained in the experimental group without artificial infestations, as sentinels of natural infestations. During the experiment, air temperature averaged 21.2 °C, ranging from 8.9 °C to 32.2 °C, and relative air humidity averaged 72.4%, ranging from 25.5% to 97.5%. At the beginning of the infestations, heifers weighed 370.1 ± 44.6 kg.

2.3. Tick collection

From the 19th through the 23rd day after each infestation, engorged females with at least 4.5 mm of diameter were daily counted on the left side of each animal (Wharton and Utech, 1970). Ticks were manually collected on the day of highest observed burden (22nd day after the first infestation and 21st day after the others). Sampled ticks from each heifer were placed in individually identified containers and brought to the Parasitic Diseases Laboratory at the Federal University of Pelotas, in the city of Capão do Leão/RS, Brazil. Here, tick's initial weight, egg mass weight and residual weight were recorded and egg production and nutrient indexes calculated.

At the laboratory, ticks were washed with distilled water, dried using absorbent paper and placed in a recipient. Due to high infestation levels presented by susceptible heifers, evaluations were restricted to a maximum of ten randomly selected ticks per heifer in each infestation. For sampling, each engorged female was blindly removed from the recipient, and before the next withdrawal the pool of ticks of each heifer was mixed again. The selected engorged females were individually weighed, placed in petri dishes and incubated at 27 ± 1 °C and relative humidity at 80 ± 10% for oviposition.

Fourteen days after incubation, the mass of eggs produced by each female tick was weighed. Starting at the second tick sampling, residual tick weights were also registered after the oviposition period. The egg production index (EPI) and nutrient index (NI) were determined according to Bennett (1974), using the following formulae:

$$\mathrm{EPI}\,(\%) = \frac{\mathrm{EW} \times 100}{\mathrm{IW}}$$

$$\mathrm{NI}\,(\%) = \frac{\mathrm{EW} \times 100}{\mathrm{IW} - \mathrm{RW}}$$

where IW is the tick initial weight, EW is the egg mass weight, and RW is the residual tick weight.

2.4. Statistical analyses

Tick biological traits and count data were analyzed by repeated measures analysis of variance using the MIXED procedure of the SAS software (SAS Institute Inc., 2008). To satisfy the normal distribution assumption, egg production index and nutrient index data were transformed by arcsine and tick count data by $\log_{10}$ (count + 1). The statistical model used was:

$$Y_{ijk} = \mu + G_i + T_j + H_{k(i)} + (G \times T)_{ij} + e_{ijk},$$

where Y_{ijk} is the tick count or average biological trait of ticks obtained from the kth heifer within the ith resistance group in the jth time of infestation; μ is the overall mean; G_i is the tick resistance group effect; T_j is the infestation time effect (1–4); $H_{k(i)}$ is the heifer random effect within the ith resistance group; $(G \times T)_{ij}$ is the interaction between tick resistance group and infestation time; and e_{ijk} is the random experimental error.

Comparisons of mean differences were based on Tukey–Krammer test ($P < 0.05$). Spearman's correlation coefficients between tick count breeding values and the number of ticks registered in the 40 heifers after natural and artificial infestations were estimated. For this correlation analysis, multiple counts on the same animal were averaged and transformed using the $\log_{10}$ function. Spearman's correlation coefficients were further used to verify associations among tick biological traits within resistance group, and between tick count breeding values and tick biological traits. The relationship between tick initial weight and egg mass weight was evaluated using linear regression analysis. To investigate the potential level of tick environmental spread, average tick count of each heifer was multiplied by the average egg mass weight from engorged ticks collected on their bodies. A two sample Student's *t*-test was performed to verify the resistance group effect on the total egg mass produced by each heifer. For this analysis, the total egg mass was transformed using the $\log_{10}$ function. All the statistical analyses were performed using the SAS software (SAS Institute Inc., 2008).

3. Results

3.1. Breeding values and tick counts

Means (±SD) of predicted breeding values for tick counts were −0.17 ± 0.07 for the resistant group and 0.17 ± 0.05 for the susceptible group. Untransformed means of tick counts after each infestation registered on the days of tick collection are represented in Fig. 1. The experimental group showed a highly significant effect ($P < 0.0001$) on the tick counts. Tick counts on susceptible heifers were 5.5, 10.5, 11.1 and 6.9 times larger than on resistant heifers, respectively, after the first, second, third and fourth artificial infestations.

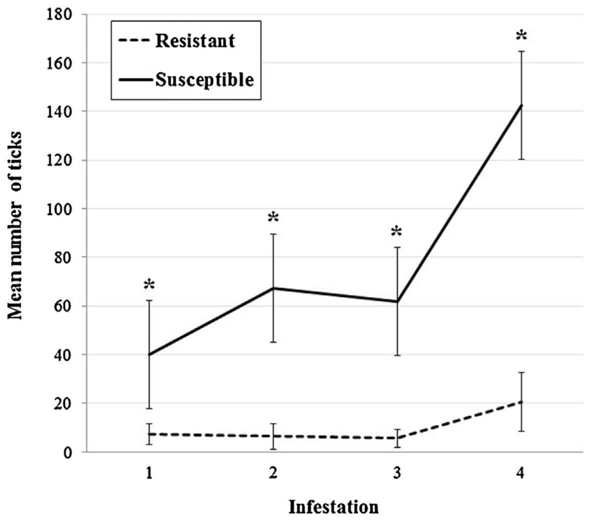

Fig. 1. Mean and standard error bars for tick counts according to infestation number and genetic tick resistance group. Asterisks indicate difference between resistant and susceptible heifers ($P < 0.0001$).

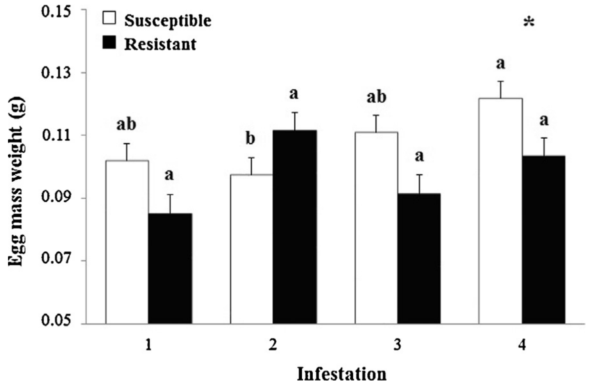

Fig. 2. Least square mean and standard error bars of egg mass weight from engorged ticks from genetically tick-resistant and tick-susceptible Braford heifers over four artificial infestations. Different small letters indicate difference ($P < 0.05$) within the same tick resistance group between artificial infestations. Asterisks indicate difference ($^{*}P < 0.05$) between resistant and susceptible groups within a specific infestation. Number of heifers that provide engorged ticks at first, second, third and fourth infestation used in these evaluations was, respectively, nine, three, five and 12 for tick-resistant group, and 17, 15, 14 and 19 for tick-susceptible group.

Correlations between tick count breeding values and tick counts after natural and artificial infestations were, respectively, 0.91 and 0.63 ($P < 0.0001$). Correlation between tick counts presented by the 40 infested heifers under natural and artificial infestations was 0.72 ($P < 0.0001$).

3.2. Natural infestations in sentinel animals

Mean (±standard error) tick counts of the resistant heifers not exposed to artificial infestation were 5.5 ± 4.5, 4.5 ± 2.8 and 0.5 ± 0.4 after the first, second and third infestation, respectively. The susceptible heifer not exposed to artificial infestation presented, respectively, 9.0, 17.0 and 3.0 ticks after the first, second and third infestation. No ticks were observed in resistant and susceptible sentinel heifers after the fourth infestation.

3.3. Tick biological traits

There was no difference ($P > 0.05$) between tick resistance groups for tick initial weight and residual weight, with respective means (95% confidence interval) of 0.204 g (0.186–0.222) and 0.044 g (0.038–0.050) for engorged ticks from resistant heifers, and 0.202 g (0.190–0.214) and 0.045 g (0.041–0.049) for engorged ticks from susceptible heifers. There was significant interaction between resistance group and infestation time ($P < 0.05$) for egg mass weight (Fig. 2), egg production index (Fig. 3) and nutrient index (Fig. 4). In the third infestation, ticks from resistant heifers showed lower egg production index ($P < 0.0001$) than ticks from susceptible heifers. In the fourth infestation, ticks from susceptible group showed higher egg mass weight ($P < 0.05$) and nutrient index ($P < 0.0001$) than ticks from resistant heifers.

Spearman correlation coefficients among tick traits are presented in Table 1. Tick initial weight was positively associated with egg mass weight and residual weight for both resistance groups. On the other hand, egg production index and nutrient index correlations with initial weight, egg mass weight and residual weight were substantially different for susceptible and resistant heifers. In the susceptible group, a positive correlation between tick initial weight and egg production index was registered, while in the resistant group the association between the same traits was negative. Despite the positive correlations between initial weight and egg mass weight in both groups, we verified that the egg mass weight regression slope on initial weight of ticks from resistant heifers was of smaller magnitude compared to that observed with engorged ticks from susceptible heifers (Fig. 5). No relationship was found between egg production index or nutrient index and initial weight, egg mass weight and residual weight in ticks

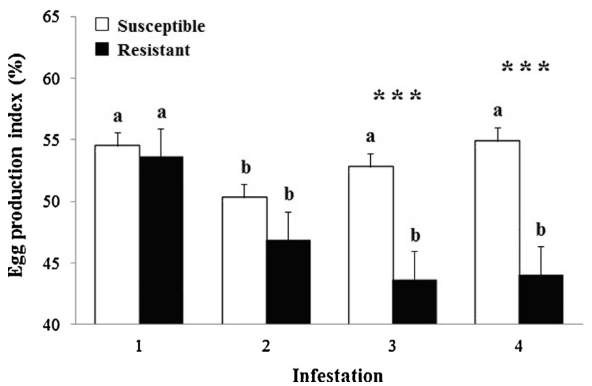

Fig. 3. Least square mean and standard error bars of egg production index from engorged ticks from genetically tick-resistant and tick-susceptible Braford heifers over four artificial infestations. Different small letters indicate difference ($P < 0.05$) within the same tick resistance group between artificial infestations. Asterisks indicate difference ($^{***}P < 0.0001$) between resistant and susceptible groups within a specific infestation. Number of heifers that provide engorged ticks at first, second, third and fourth infestation used in these evaluations was, respectively, nine, three, five and 12 for tick-resistant group, and 17, 15, 14 and 19 for tick-susceptible group.

Table 1
Correlation among engorged tick biological traits obtained from genetically tick-resistant and tick-susceptible artificially infested Braford heifers.

Trait	Group	Initial weight (g)	Egg mass weight (g)	Residual weight (g)	Egg production index (%)
Egg mass weight (g)	Susceptible	0.97***			
	Resistant	0.80***			
Residual weight (g)	Susceptible	0.89***	0.85***		
	Resistant	0.62**	NS		
Egg production index (%)	Susceptible	0.32*	0.50***	0.39**	
	Resistant	−0.46*	NS	NS	
Nutrient index (%)	Susceptible	0.59***	0.66***	0.64***	0.72***
	Resistant	NS	NS	NS	0.81***

NS: not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.0001$.

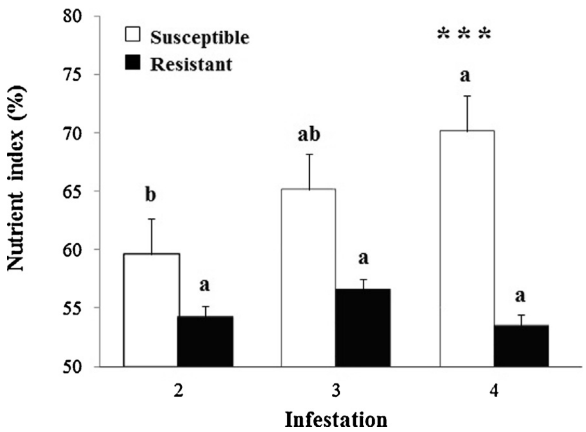

Fig. 4. Least square mean and standard error bars of nutrient index from engorged ticks from genetically tick-resistant and tick-susceptible Braford heifers over artificial infestations. Different small letters indicate difference ($P < 0.05$) within the same tick resistance group between artificial infestations. Asterisks indicate difference (*** $P < 0.0001$) between resistant and susceptible groups within a specific infestation. Number of heifers that provide engorged ticks at second, third and fourth infestation used in these evaluations was, respectively, three, five and 12 for tick-resistant group, and 15, 14 and 19 for tick-susceptible group.

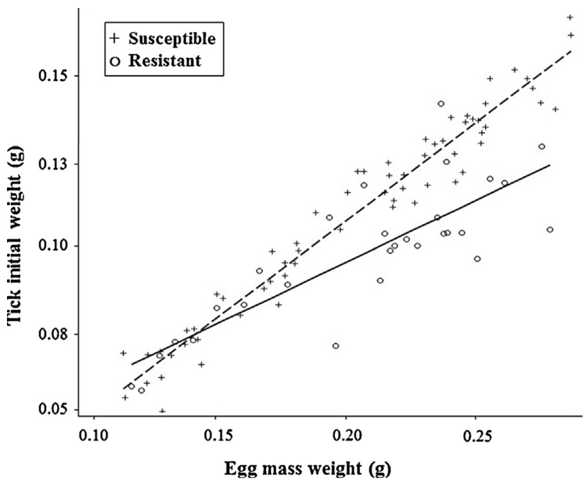

Fig. 5. Egg mass weight as a function of the initial weight of engorged ticks from genetically tick-susceptible and tick-resistant Braford heifers.

obtained from resistant heifers. For females engorged on susceptible heifers, all these tick weight traits were in positive association with egg production index and nutrient index. Analyses of correlations between tick count breeding values and tick biological traits showed significant associations with egg mass weight ($r = 0.20$; $P = 0.05$), egg production index ($r = 0.28$; $P < 0.01$) and nutrient index ($r = 0.40$; $P < 0.0001$). The total egg mass weight of ticks from susceptible heifers (2.88 g) was higher ($P = 0.001$) than the mean observed in resistant heifers (1.35 g).

4. Discussion

The utility of genetic evaluations based on natural infestations to classify cattle as tick-resistant or tick-susceptible was demonstrated by the remarkably lower tick burden observed in the resistant group after artificial infestations. The positive associations between tick count breeding values and tick counts after natural and artificial infestations indicate that tick burdens are similar in both challenge methods and confirm that breeding values for tick counts are useful to predict tick resistance in cattle.

Considering the relatively low tick counts registered on heifers that were not exposed to artificial infestations, it is reasonable to assume that the number of ticks recorded on artificially challenged heifers was not significantly influenced by possible natural infestations. Exposure of uninfested heifers to facilities where infested heifers received the ticks larvae has probably caused the development of some ticks on sentinel animals.

Development of tick resistance over successive infestations was shown by Hewetson (1968) in Sahiwal and Illawara Shorthorn cattle, and by Wagland (1975) in Brahman and Shorthorn cattle. They observed that tick initial weight decreased with the development of resistance by naïve animals. However, in the present study with short intervals between infestations and non-naïve heifers, there was no influence of infestation time on tick initial weight.

An increasing egg mass weight mean was observed from the second to the fourth infestation on susceptible heifers, while genetic resistance may have provided a defense mechanism to resistant heifers that maintained egg mass relatively constant over the four infestations (Fig. 2). Considering that the average egg mass weight difference between ticks from resistant and susceptible heifers over the four infestations (Fig. 2) was 0.012 g and that 1 g of *R.* (*B.*) *microplus* egg mass is about 20 thousand eggs (Drummond et al., 1973; Sutherst et al., 1978; Gonzales, 1993), each engorged tick from the susceptible group produced 240 eggs (12%) more than engorged ticks from resistant heifers. Added to the fact that susceptible hosts carry a larger number of ticks (Fig. 1), this indicates that genetic resistance may also contribute to reduce tick infestation levels on pastures. Consequently, gradual culling of tick-susceptible animals could be used as an alternative to reduce environmental tick populations, decreasing constant acaricide treatments and other damages caused by parasitism (Sutherst et al., 1979; Madalena et al., 1985).

Egg production index and nutrient index estimate the tick efficiency in converting nutrients into eggs. The ability of resistant heifers to reduce egg mass seems to be related to depletion of nutrient utilization. Smaller egg production index from the third infestation (Fig. 3) and smaller nutrient index in the fourth infestation (Fig. 4), registered in the resistant group, indicates that ticks collected from resistant heifers converted ingested blood to egg mass less efficiently than ticks from the susceptible group. Therefore, the defense mechanisms presented by resistant animals in response to infestations, besides being detrimental to larvae development and reducing the number of adult parasites, apparently also affected *R.* (*B.*) *microplus* physiological processes involved in conversion of tick initial weight to mass of eggs.

High correlations between tick initial weight and egg mass weight (Table 1) suggest that both traits were affected by similar host immunological factors, agreeing with results presented by Barriga et al. (1995). Nearly perfect association ($r = 0.97$) between those traits in susceptible heifers indicates that egg mass produced by ticks of this group were almost entirely dependent on blood intake capacity during tick development on the cattle. Although also positive and high, the correlation of 0.80 observed between initial weight and egg mass weight for resistant heifers indicates that a small portion of the variation observed in oviposition weight was due to factors other than tick initial weight in this group. The lower correlation between tick initial weight and egg mass weight observed in the resistant group, as well as the negative association between initial weight and egg production index of ticks from resistant heifers, may be an indicative of occurrence of a defense mechanism that disturbs conversion of ingested blood to egg mass in animals of the resistant group.

Positive correlations observed between tick count breeding values with egg mass weight, egg production index and nutrient index suggest that genetic evaluation and selection of animals classified as tick-resistant may, in the long term, influence not only the number of ticks that develop on cattle, but also reduce parasite dissemination in the environment and infestation levels in the field. Higher mean of total egg mass weight of ticks from susceptible heifers point out that the observation of reduced tick number would not necessarily lead to reduction in total egg production if there is a corresponding increase in egg mass weights.

In conclusion, genetic variability for tick resistance observed in Braford heifers affected both the number of ticks carried by the animals as well as egg mass weight, egg production index and nutrient index of engorged females of *R.* (*B.*) *microplus*. Thus, in addition to economic losses related to reduction of cattle productivity or to higher demand for treatments, maintaining animals with high tick susceptibility in the herd implies higher environmental infestation levels, perpetuating high prevalence of ticks on farm. Genetic evaluation of livestock for tick resistance and inclusion of tick counts as selection criteria in breeding programs can be implemented as an auxiliary tool for the strategic control of *R.* (*B.*) *microplus* in production systems. Finally, is important to note that further studies to uncover genetic factors responsible for immunological mechanisms involved in the expression of resistant phenotypes could enable genetic evaluations for tick resistance based on genomic information (e.g., molecular markers); thereby, increasing prediction accuracy, accelerate identification of superior genotypes, and avoid cattle exposure to ticks required by current genetic evaluation methods.

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